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Abstract: Background Topographical modifications of the surface influence several cell functions and can be exploited to modulate cellular activities such as adhesion, migration and proliferation. These complex interactions are cell-type specific, therefore engineered substrates featuring patterns of two or more different topographies may be used to obtain the selective separation of different cell lineages. This process has the potential to enhance the performance of biomedical devices promoting, for example, the local coverage with functional tissues while demoting the onset of inflammatory reactions. Findings Conclusions Here we present a computational tool, based on Monte Carlo simulation, which decouples the contribution of cell proliferation and migration and predicts the cell-separation performance of topographically engineered substrates. Additionally, we propose an optimization procedure to shape the topographically engineered areas of a substrate and obtain maximal cell separation.

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REPORT

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On cell separation with topographically engineered surfaces

Davide Franco^{1,3}, Mirko Klingauf^{1,2}, Marco Cecchini², Volkmar Falk³, Christoph Starck³, Dimos Poulikakos¹ and Aldo Ferrari^{1*}

Abstract

Background: Topographical modifications of the surface influence several cell functions and can be exploited to modulate cellular activities such as adhesion, migration and proliferation. These complex interactions are cell-type specific, therefore engineered substrates featuring patterns of two or more different topographies may be used to obtain the selective separation of different cell lineages. This process has the potential to enhance the performance of biomedical devices promoting, for example, the local coverage with functional tissues while demoting the onset of inflammatory reactions.

Findings & Conclusions: Here we present a computational tool, based on Monte Carlo simulation, which decouples the contribution of cell proliferation and migration and predicts the cell-separation performance of topographically engineered substrates. Additionally, we propose an optimization procedure to shape the topographically engineered areas of a substrate and obtain maximal cell separation.

Keywords: Topography; Biomaterials; Cell migration

Findings

Introduction

Engineering micro or nanoscale topographies to the surface of biomedical devices represents a promising strategy to improve their integration in the human body and, in general, to enhance their performance [1-4]. As demonstrated both *in vivo* and *in vitro*, topographically-engineered surfaces have the potential to interfere with cell signaling cascades thereby influencing several cellular processes such as adhesion, migration and proliferation [5].

An interesting application of topographic texturing was reported by Csaderova et al. [6]. In their study, a patterned area was implemented to the surface of a biodegradable material to induce selective cell separation. Specifically, the authors manufactured a substrate featuring a central squared nanopattern surrounded by a flat surface. The topography locally promoted the population by endothelial cells which formed a continuous monolayer while

strongly demoted the growth of fibroblasts. Based on these results, the authors envisioned the use of such topographic modification on stents or vascular grafts to allow a fast re-endothelialization of the implant and minimize the risk of inflammatory reactions [7,8].

In the above-mentioned study the individual contributions of cell migration and proliferation to the separation effect were not analyzed and the final result was reported as an undivided event. However, to further understand the biological mechanisms behind cell separation the two cellular activities must be decoupled. Importantly, the contribution of proliferation only depends on the local properties of the substrate and is thus independent from the shape of the two areas. On the other hand when cells move faster on one of the two surfaces, boundary crossing results into a change of velocity and thus into a net cell flow. In this case, geometrical characteristics of the two areas, such as the border perimeter, may play a critical role since the larger is the perimeter of the nanopatterned area, the higher is the net cell flow through the interface.

Several studies investigated the interaction of mammalian cells with topographically engineered substrates and characterized the influence of the pattern on cell migration or proliferation individually [6-11]. All these data

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could be combined and used to assess cell separation efficiency on substrates featuring a combination of different topographies. However, implementing this analysis would substantially increase the complexity and number of required experiments. Therefore, we propose here a numerical model based on experimental data that predicts the cell separation performance for a given surface configuration. The method is based on Monte Carlo simulations and allows considering the contribution of individual cellular activities to the cell coverage of a substrate featuring two areas with different surface topography [12]. We further extend this method and propose a shape optimization based on the geometry of the patterned areas. Finally, we apply this analysis to identify within which experimental conditions (i.e. cell migration velocity and doubling time) shape optimization is meaningful.

Results

In order to validate the simulation code, we applied the experimental parameters reported in Csaderova et al. [6] and reproduced the results reported therein for the case of fibroblasts. The original substrate featured a squared flat area with lateral size of 2 cm which included a

central squared nanopatterned area with a lateral size of 1 cm (Figure 1b). The individual cell migration velocity, extracted from time-lapse sequences (Figure 1a), was set to 50 $\mu\text{m}/\text{hour}$ and 25 $\mu\text{m}/\text{hour}$ for the nanopatterned and the flat area; respectively. The doubling time was similarly set to 22 hours for cells on the flat area and to 26 hours for cells on the nanopatterned area (see methods section) [6]. In order to replicate the experimental conditions reported in [6] where both the flat and nanopatterned regions were isotropic, we assumed random cell displacement.

We simulated cell proliferation and migration up to 72 hours with time intervals of 15 minutes. The resulting cell distribution well reproduced the effect reported by Csaderova et al. [6] (compare Figure 1b with Figure 2d in Csaderova et al.). The results of our quantitative analysis of the separation effect are reported in Figure 1d-e. The relative cell density (i.e. cell density on the nanopatterned area normalized by the cell density on the flat surface) decreases over time, becoming significant after 48 hours (e). In all, the trend obtained in our model is similar to the one observed experimentally (compare Figure 1d-e with Figure 2a in Csaderova et al. [6]). Interestingly, our experiment *in silico* captured the formation of cell agglomerates

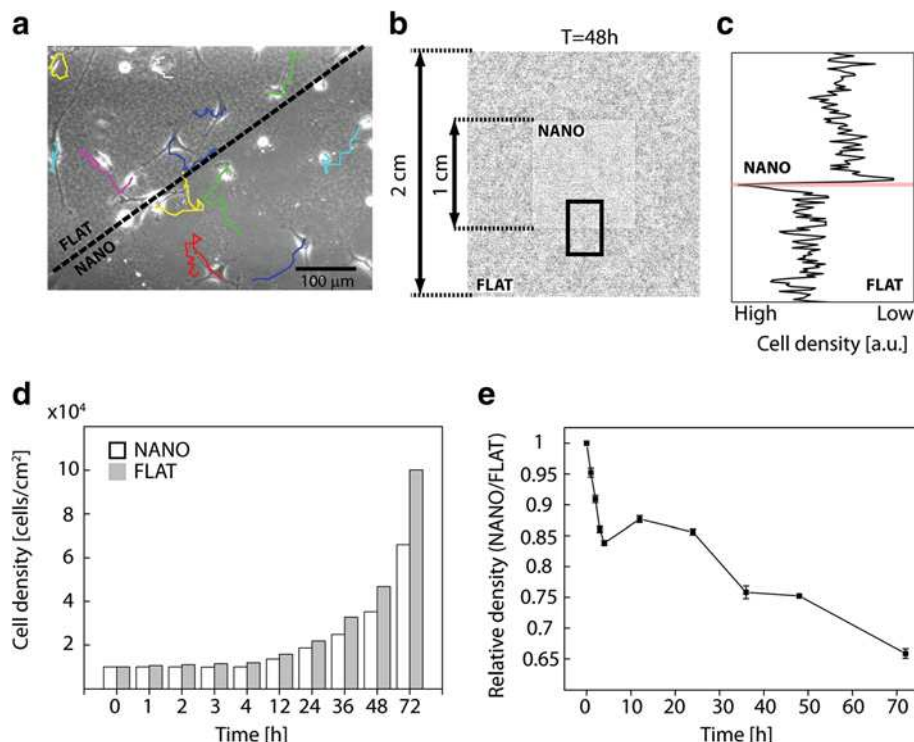


Figure 1 Monte Carlo simulations used to reproduce experimental results. (a) Cell tracks used to extract the migration velocity both on the flat (FLAT) and the nanopattern (NANO). (b) Cell distribution within a sample with a square nanopatterned area in the centre (NANO) surrounded by a flat area (FLAT) after simulation the cell growth for 48 hours. (c) Density profile along the center line of the black frame; the red line correspond to the interface between the two areas. (d) Cell density both on the nano pattern and the flat over time. (e) Relative density reported over time. The error bars represent the standard error of the mean.

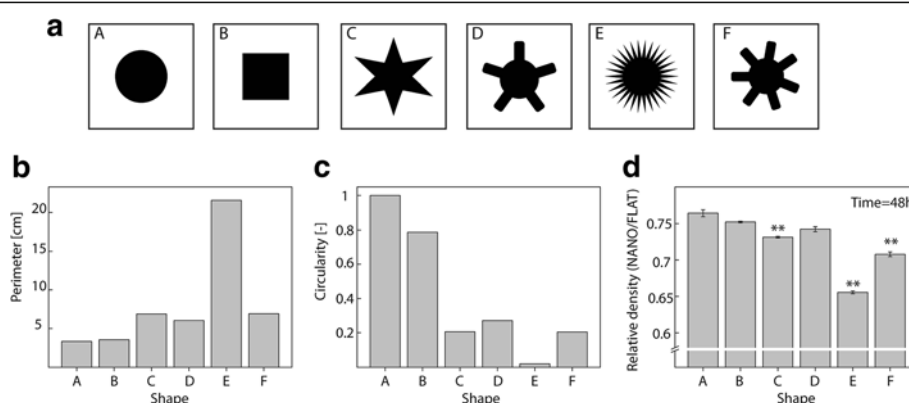


Figure 2 Cell selective growth obtained using different nanopattern shapes. (a) Tested shapes and their perimeter **(b)** and circularity **(c)**. **(d)** Relative density obtained for all the tested shapes after simulating the cell growth for 48 hours. The errors bars represent the standard error of the mean. Significant differences with the shape B are reported (**for $p < 0.01$).

on the flat surface at the interface with the nanopatterned area (compare Figure 1b with Figure 2d in Csaderova et al. [6]). Figure 1c shows the cell density profile extracted at the border between the two areas. This result can be explained assuming a significant contribution of migration to the final cell distribution.

To test this hypothesis we simulated cell proliferation and migration on substrates with different geometrical shapes (Figure 2a). In particular, shapes were chosen to have the same overall surface but different perimeter shape and length (Figure 2b and 2c). The relative cell density obtained after 48 hours is reported in Figure 2c for each of the tested configurations. In particular, we observed significant differences between the relative density obtained with the original shape (shape B) and shapes C (0.73), E (0.65) and F (0.70). These results indicate that under specific experimental conditions cell separation on a substrate can be optimized simply changing the shape of the patterned area.

We next performed a parametric study to identify under what experimental conditions shape optimization may be beneficial. For this, we varied the cell doubling time and the migration velocity on the patterned area within the physiological values reported in literature [13-16] while the relative values were kept constant for the surrounding flat area. For each condition cell growth and migration were simulated for 48 hours in order to investigate the cell separation performance of nanopatterned surfaces in the early phases upon the interaction with cells. Starting from an initial condition where a number of cells (40000) were homogeneously and randomly distributed on a substrate containing two different areas (a nanopatterned area encircled by a flat area), the cell separation performance of a substrate was obtained based on two parameters, i.e. the migration velocity and the doubling time, assigned to cells as a function of their position (i.e. nanopatterned or flat).

This simulation was run for six different configurations where the only change was in the shape of the nanopatterned area. All other parameters were unaltered.

We introduced C_s as a parameter to describe the eventual improvements in the cell separation performance obtained by purely changing the shape of the nanopatterned area. Specifically, the relative cell density values (cell density on nanopattern/cell density on flat) at 48 h for each of the six different configurations (from A to F) were obtained. The parameter C_s was calculated as the ratio between the highest and the lowest relative cell density obtained among the tested configurations. Thus, high C_s indicates a strong impact of shape optimization on cell separation (Figure 3).

The colorimetric map in Figure 3 clearly shows how C_s is affected by the migration velocity (horizontal axis) rather than by the doubling time (vertical axis). Additionally, we envision that full cell separation (i.e. cell coverage exclusively on the external flat area), can be obtained only when the difference in cell migration velocity between the two areas is significant. In this case, cell separation would be mostly obtained through cell displacement, and the contribution of cell proliferation could be neglected. Altogether, these results confirm that the shape optimization may be beneficial only on substrates where cell separation is predominantly driven by cell migration.

Conclusion

In summary, we have presented a numerical method based on Monte Carlo simulation to model cell separation obtained using a specific topographic pattern (Figure 1). We investigated how cell migration and proliferation individually contribute to the separation effect and we demonstrated that the geometrical shape characteristics of the patterned area have the potential to modulate cell separation. As an example, we tested six different shapes with

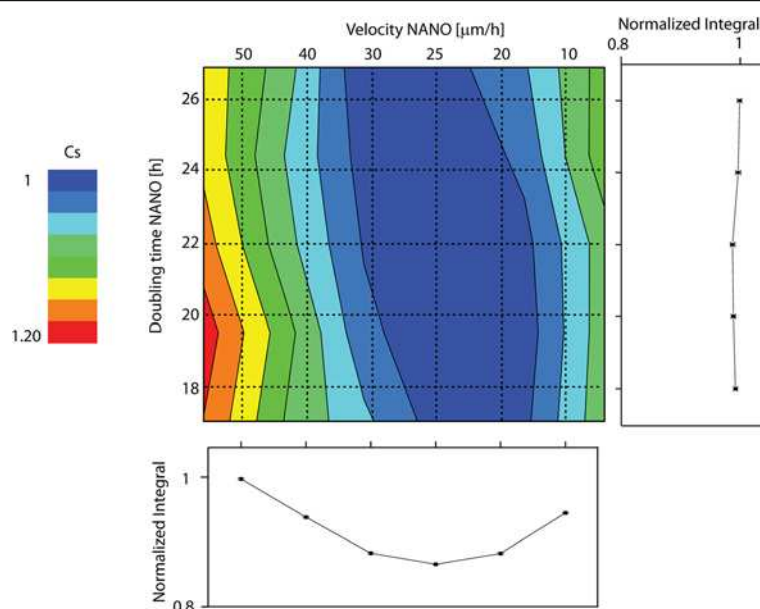


Figure 3 Influence of the doubling time and migration velocity on the shape optimization. The color map shows the separation parameter calculated with the relative density obtained after simulating cell growth for 48 hours using different migration velocity and doubling time.

the same area but increasing perimeter. When cell separation is predominantly driven by a difference in migration between the two areas, the shape with the longest perimeter provides the best separation performance (Figure 2). Finally, we have parameterized the experimental conditions in which shape optimization may be beneficial (Figure 3). This study will assist the design of optimized substrates featuring enhanced cell separation.

Methods

Monte Carlo simulations, used to mimic cell growth, were implemented using the C++ programming language. The code was adapted from what reported in Klingauf et al. [12]. 40000 cells were initially seeded with random distribution on each sample as reported by [6]. The total area of the sample was 4 cm², while the nanopatterned area was always 19.4 ± 0.1% of the total area independently from the shape assigned to the nanopatterned area (Figure 2).

The cells were treated as solid spheres and therefore no overlap was allowed. The minimal distance allowed between neighbouring cell centres was set to 10 μm. This value was chosen to fit the average measured cell body diameter. In the case in which a new cell was assigned within the domain of an existing cell, the code was attempting a new assignment until a free space was obtained.

Two input parameters were specified to run the simulations: Doubling time and migration velocity. The migration

velocity of fibroblasts contacting the flat or the nanopatterned area was extracted from time-lapse movies using the manual cell tracker tool of ImageJ (National Institute of Health, USA). Experimental data were kindly provided by Dr. Mathis Riehle (Institute of Molecular, Cell and Systems Biology, University of Glasgow). The velocity value extracted were 50 μm/hour and 25 μm/hour for the patterned area and the flat respectively. The migration velocity was always implemented with a standard deviation of 10% of the nominal value. The doubling time was set to 22 hour for the flat (according to our previous studies [16]), while for the nanopatterned area was tuned to match the experimental trend observed in Csaderova et al. and thus set to 26 hours.

At the beginning of the simulation 40000 cells were randomly seeded over the entire surface which is composed by a nanopatterned surrounded by a flat area. These initial settings reproduce the experimental settings in [6]. At this point a “doubling time state” was randomly assigned to each cell. This random assignment was set to reproduce a condition in which the cell cycle is not synchronized. This is to be expected for both the *in vitro* tests we refer to and the related physiological conditions. The *doubling time state* ranges linearly from zero (corresponding to the time step at which the cell is dividing) to a maximum value corresponding the number of time steps till next division (which is retrieved from the doubling time, in hours, set for the corresponding area of the substrate times the number of time

steps simulated per hour). At every time step (corresponding to 15 minutes) the code updates the time-state of each cell (*doubling time state* of previous time point + 1). Cells reaching the maximum *doubling time state* (for example for cells on the flat area the maximum *doubling time state* is set to $22 \times 4 = 88$) will divide. This means that in the surrounding of the dividing cell (mother cell) a new cell (daughter cell) will be introduced and placed in a free space. The code will additionally reset the *doubling time state* of both the daughter and the mother cell to zero in order to start a new cycle.

The daughter cells are seeded in the available free space within the surroundings of the mother cell (within 10 μm). Therefore, the assignment is not random but related to the original position of the doubling cell.

For the parametric study reported in Figure 3 the doubling time and the migration velocity on the patterned area were set within the range of 18 hours to 26 hours and of 10 $\mu\text{m}/\text{hour}$ to 50 $\mu\text{m}/\text{hour}$ respectively. In contrast, on the flat area the assigned values were constantly set to 22 hours and 25 $\mu\text{m}/\text{hour}$, respectively.

In our simulation the cell movements followed a random walk model without any preferential directionality. This model was chosen since the substrates considered do not provide any directional information and must be considered as fully isotropic.

Each Monte Carlo step simulates the cell displacement within 15 minutes. This resolution was chosen for two reasons. First, to match the experimental settings used to acquire the movies of cell migration we refer to. In turn, a higher sampling of the cell position would introduce a 'biological noise' effect, by which minor cell shape changes generated by membrane deformation (and thus not to be accounted as active cell migration) would artificially increase the measured cell velocity.

The colour map in Figure 3 reports the C_s coefficient obtained starting from different simulation parameters within a range that was chosen to include all reported values in literature [13-16]. The normalized integral values reported on the sides of the map in Figure 3 were calculated using the C_s values depicted in the colour map. Specifically, the five points in the right side graph represent the average C_s values obtained for each of the set doubling times (18, 20, 22, 24, 26 hours), while the six points in the bottom side graph represent the average C_s values obtained for each migration velocity set (50, 40, 30, 25, 20, 10 $\mu\text{m}/\text{hour}$).

Since Monte Carlo simulations are based on pseudo-random processes, each simulation was repeated three times. Hence, the values reported represent the average among the results of three simulations. Statistical comparison of average relative density obtained with different nanopattern shapes was performed using a Student's

t-test. All values reported are expressed as average \pm the standard error of the mean.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

DF performed the simulations, conducted the data analysis and wrote the manuscript. MK performed the simulations and wrote the simulation code. MC and DP co-supervised the work and edited the manuscript. AF devised the project, supervised the work and edited the manuscript. All authors read and approved the final manuscript.

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